Calcium carbonate intercalated Starch methacrylate and N-cyclohexylacrylamide based Hydrogels: Synthesis, Antioxidant and Antimicrobial activities

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Abstract

In the present study, calcium carbonate intercalated Starch Methacrylate and N-cyclohexyl acrylaminde hydrogels were synthesized by free radical polymerization in Methanol/water medium at 65°C. The Starch methacrylate monomer (SMA) was prepared by esterification reaction. AIBN initiator and MBA crosslinler are used and the $CaCO_3$ was intercalated via in situ polymerization. The synthesized nanocomposites are characterized by FTIR, SEM and TGA analysis. These hydrogels were subjected to investigate anti-oxidant and anti-microbial activities. The results reveal that the nanocomposite materials are biologically important one.

Keywords: Starch methacrylate; FTIR spectroscopy; SEM analysis; Antioxidant; Antimicrobial activity

1. INTRODUCTION

Hydrogels are 3D polymeric materials, which absorbs large quantity of water and are insoluble in water, these materials are useful for drug delivery, scaffold for tissue engineering, drug carriers and supercapacitors. Compared with other types of biomaterials, hydrogels have the advantages of increased biocompatibility, tunable biodegradability, mechanical strength and porous structure. However, due to the low mechanical strength and fragile nature of the hydrogels, the feasibility of applying hydrogels is still limited. Thus, novel hydrogels with stronger and more stable properties are still needed and remain an important direction for research. Starch based hydrogels and Calcium carbonate nanoparticles are biocompatible, biodegradable and low cost for its application in various fields.

Inorganic nanoparticles for biomedical applications have undergone extensive investigations in recent years. Among different inorganic drug carriers, calcium carbonate ($CaCO_3$) nanoparticles showed unique advantages due to their ideal biocompatibility and the potential as delivery system for loading different categories of drugs. The accessibility, low cost, safety, biocompatibility, pH-sensitive properties, conductivity and slow biodegradability of $CaCO_3$ particles nominate it to be a suitable for drug delivery carrier. [1, 2].$CaCO_3$NPs do not affect the cell viability of normal (NIH 3T3) and cancer (MCF7) cells. High concentrations of $CaCO_3$NPs exhibit no genotoxicity against NIH 3T3 and MCF7 cells. $CaCO_3$NPs have no developmental toxicity to zebrafish embryos. [3]

XiangliRu et al.,[4] studied the $CaCO_3$ functionalized erythrocytes which are useful to remove extracellular Lead ions upto 80%. Therefore it is believed to be a potential material to reduce the Pb^{2+} level in kidney and liver. Shadpour Mallakpour et al., also reported the removal of Heavy metal ions using $CaCO_3$ Nanoparticles containing tragacanth gum materials [5]. Hydrogels derived from the Arabinoxylan natural polymer is functionalized carboxymethyl group and loading of reduced graphine nanosheets to study the skin cancer treatment using fluorouracil drug [6].

The naturally occurring starch based monomers copolymerized with some N-substituted acrylamide monomer [7]. The authors studied the electrical conductivity of polymer as a function of temperature and results showed that these polymers showed semiconducting behavior and optical properties. Polymers with excellent absorption properties were synthesized by graft polymerization: soluble starch-g-poly(acrylic acid-co-2-hydroxyethyl methacrylate), poly(vinyl alcohol)/potato starch-g-poly(acrylic acid-co-acrylamide), poly(vinyl alcohol)/potato starch-g-poly(acrylic acid-co-acrylamide-co-2-acrylamido-2-methylpropane sulfonic acid). Ammonium persulfat e and potassium persulfate were used as initiators, while N,N′-methylenebisacrylamide was used as the crosslinking agent. The molecular structure of potato and soluble starch grafted by synthetic polymers was characterized by means of Fourier Transform Infrared Spectroscopy (FTIR). The absorption properties of the obtained biopolymers were tested in deionized water, sodium chromate solutions of various concentrations and in buffer solutions of various pH [8].

Grafting of N-cyclohexylacrylamide (NCA) with cellulose methacrylate monomer used to study the metal ion and water uptake properties at 250ppm of Ni^{2+} / Co^{2+} / Cu^{2+} / Pb^{2+} / Fe^{3+} / Cr^{3+} [9]. Hossam et al., reported the synthesis of carboxymethyl cellulose / acrylic acid gel via electron beam irradiation with (1.5 Mev& 25 KW) electron beam [10].Sukriti B.S. Kaith described the synthesis of Gum Xanthan grafted with polyacrylic acid in the presence of Glutaraldehyde (crosslinker) and APS initiator. [11]. Seidy Partrose Santose et al., [12] described that the starch based hydrogels and Chitosan loaded nanocomposite systems shows relevant properties for tissue engineering. The synthesized starch-chitosan hydrogels had 80% cell viability towards HEP-2 (Human epidermal type 2 cells) on mice. Based on the literature, we planned to synthesize $CaCO_3$ NPs intercalated Starch based Nanocomposite Hydrogels to investigate anti-oxidant and antimicrobial activities.

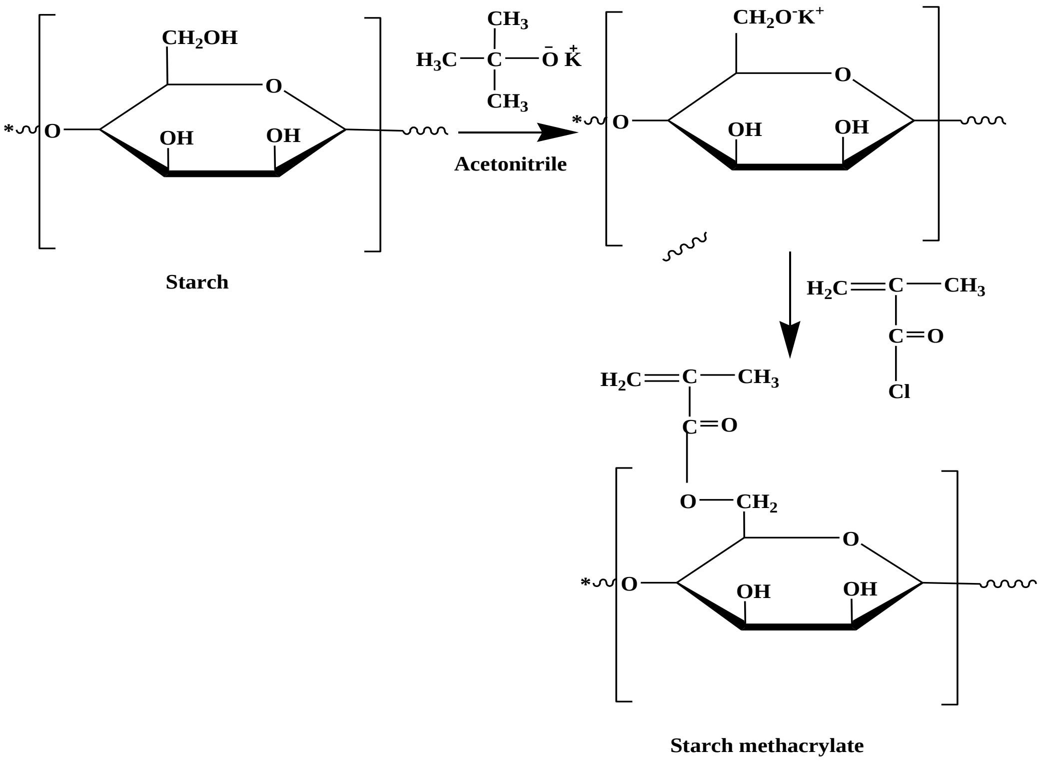
2. EXPERIMENTAL

2.1. Materials

Starch, AIBN, acetonitrile, Calcium carbonate NPs and Acrylonitrile were purchased from SD–fine chemicals Limited in India. The N-cyclohexylacrylamide **(NCA)** monomer is synthesized by the reaction of Acrylonitrile and Cyclohexane in the presence of H_2SO_4 at 0°C [13].

2.2. Starch-Methacrylate Monomer (SMA)

The soluble form of starch treated with methacryloyl chloride in drops and refluxed for 8 hrs at room temperature (Scheme-1).



Scheme 1: Synthesis of Starch-Methacrylate monomer

Synthesis of $CaCO_3$Nanocomposite hydrogels

The starch based $CaCO_3$Nanocomposite hydrogels are synthesized by free radical polymerization at 65°C. The required amount of SMA and NCA are dissolved in methanol: water (3:1) medium and AIBN initiator, MBA crosslinker along with purging of nitrogen gas for 30 minutes before the polymerization. The polymerization time is varied to prepare the $CaCO_3$ Nanoparticle intercalated nanocomposite hydrogels.

2.3. Characterization

The FTIR spectral characterization was made using Perkin Elmer 200 spectrophotometer in the 4000 to 450 cm-1 wave number range. The XRD pattern and the crystallinity of hydrogel was studied by XRD SHIMADZU instrument. The surface morphology of the starch based hydrogels are studied by JEOL JSMLV scanning electron microscope . NETZCH STA 250 thermal analyzer is used to determine the thermal stability if the polymeric material.

2.4. Antioxidant Activity of Starch based Hydrogels

The assessment of the free radical scavenging capacity of GNH was conducted using DPPH **[14].** DPPH solution (0.004%w/v) was prepared in DMSO. GNH was mixed with DMSO to create the stock solution with a concentration of 10mg/100mL or 100µg/mL. This solution was then distributed into five test tubes and, by means of serial dilution using the same solvent, the final volume of each test tube was adjusted to 10mL, resulting in concentrations of 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL and100 µg/mL respectively. Freshly prepared DPPH solution (0.004%w/v) was added to each of these test tubes and after a 10-minute incubation period, the absorbance was measured at 520 nm using a spectrophotometer. Ascorbic acid was employed as a reference standard and was dissolved in distilled water to create a stock solution with the same concentration of 10mg/100mL. DMSO was utilized as a blank. The percentage scavenging of the DPPH free radical was determined using the following formulae.

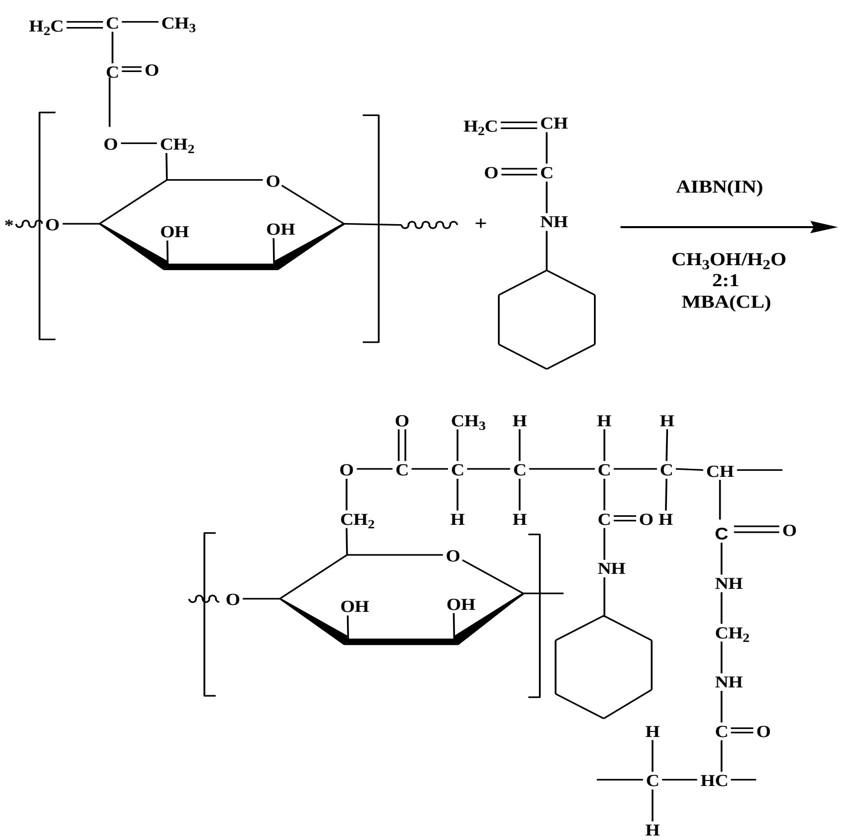
$$\%\ \text{DPPH radical scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}}\ \text{X}\ 100 \quad \text{------ (1)}$$

2.5. Antimicrobial studies of Hydrogels

In vitro antibacterial activity experiments were conducted using the fresh nutrient method to assess the impact of the synthesized polymers on microorganisms including *Staphylococcus aureus, Bacillus substilis*(Gram positive), *Escherichia coli, Salmonella paratyphi* (Gram negative) these microorganisms were selected to evaluate the antibacterial activity. Additionally,*Candidaalbicans, Aspergillus Niger, Monoscuspurpures* were employed to assess antifungal activity. To conduct the antibacterial and antifungal assays, the compounds were first dissolved in Dimethyl sulfoxide (DMSO). Subsequent dilutions of the compounds as well as standard drugs were prepared in the test medium were prepared in the test medium at concentrations of 50 and 200 ppm concentrations using fresh sabouraud's broth. The minimum inhibitory concentrations (MIC) were determined through the two fold serial dilution technique. Control drugs such as *Ciprofloxacin*and *Clotrimazole* were used. The antimicrobial activity data for the hydrogel was collected in duplicate, the data as MIC values were expressed in ppm.

3. RESULTS AND DISCUSSION

The starch based $CaCO_3$Nanocomposite hydrogels are synthesized by free radical polymerization at 65°C using SMA and NCA monomers in methanol: water (3:1) medium and AIBN initiator, MBA crosslinker. the $CaCO_3$ Nanoparticle intercalated via in situ polymerization (scheme 2).



Scheme 2: Synthesis of poly (Starch methacrylate-co-NCA) $CaCO_3$Nanocomposite Hydrogel

3.1. FTIR spectral studies of Hydrogels

The FTIR spectrum of starch based $CaCO_3$ Nanocomposite is depicted in Fig.1 and the assignments of characteristic peak values are given as table1.

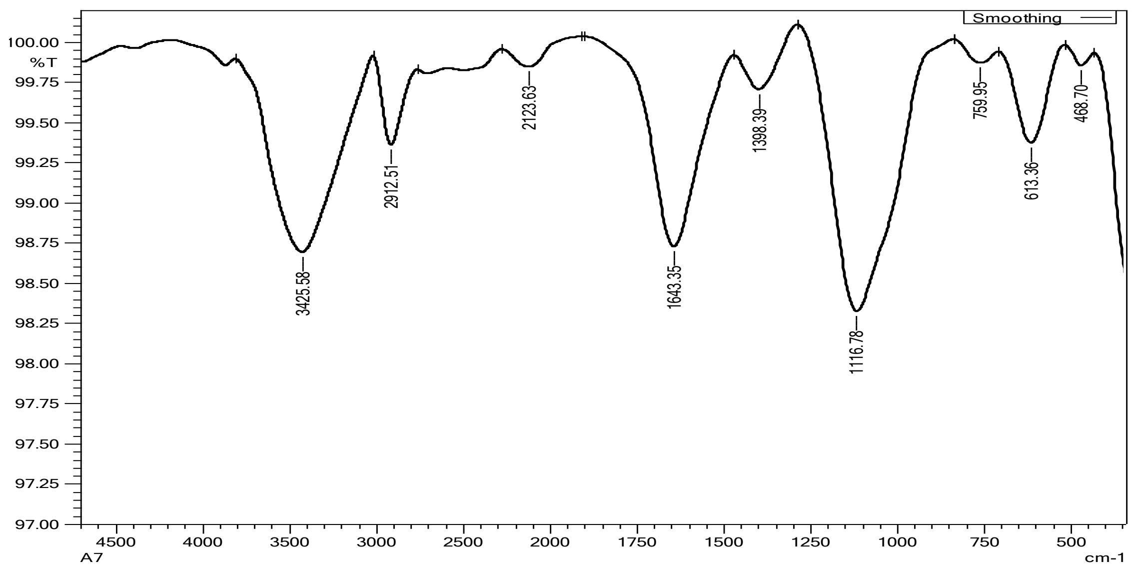


Fig.1.FTIR spectrum of poly (Starch methacrylate-co-NCA) $CaCO_3$Nanocomposite Hydrogel

Table 1. Assignment of peak values

Peaks	Functional groups
3425.58 cm^{-1}	Indicates the presence of O-H stretching vibrations of alcohols / N-Stretching vibrations for amine group
2912.51 cm^{-1}	Indicates the presence C-Hstretching vibrations bonds in alkane
2123.63 cm^{-1}	Indicates the presence C-N bond
1643.35 cm^{-1}	Indicates the presence C=Ostretching vibrations of carbonyls groups
1398.39 cm^{-1}	Indicates the presence O-Hstretching vibrations for alcohols
1116.78 cm^{-1}	Indicates the presence C-C or C-Nstretching bonds
759.95 &613.36 cm^{-1}	Indicates the presence of $CaCO_3$Nps

3.2. SEM analysis of Starch based Hydrogels

The morphology of the starch based hydogels are shown in Fig.2. with different magnification. At high magnification the morphology looks like Palm leaf structure and SEM with EDAX indicates the Calcium and Oxygen element the in the polymer matrix .

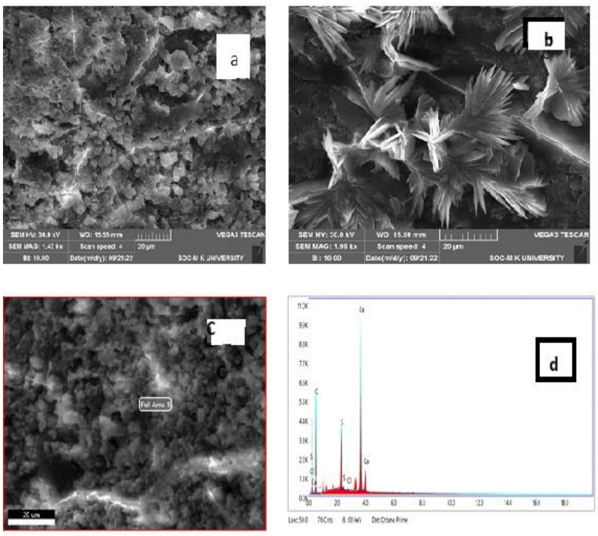


Fig.2. SEM images (a, b) in different magnification and SEM with EDAX (c, d) of Hydrogels

3.3. XRD analysis

The XRD pattern of the starch based $CaCO_3$nanocomposite hydrogels is depicted in Fig.3. A broad peak at 15-45ɵ value exhibits the hydrogel is more amorphous and less crystalline in nature. The peaks at 22.20, 28.17, 32.14 and 42.06 ɵ values confirmed the presence of nano $CaCO_3$ in the polymer matrix.

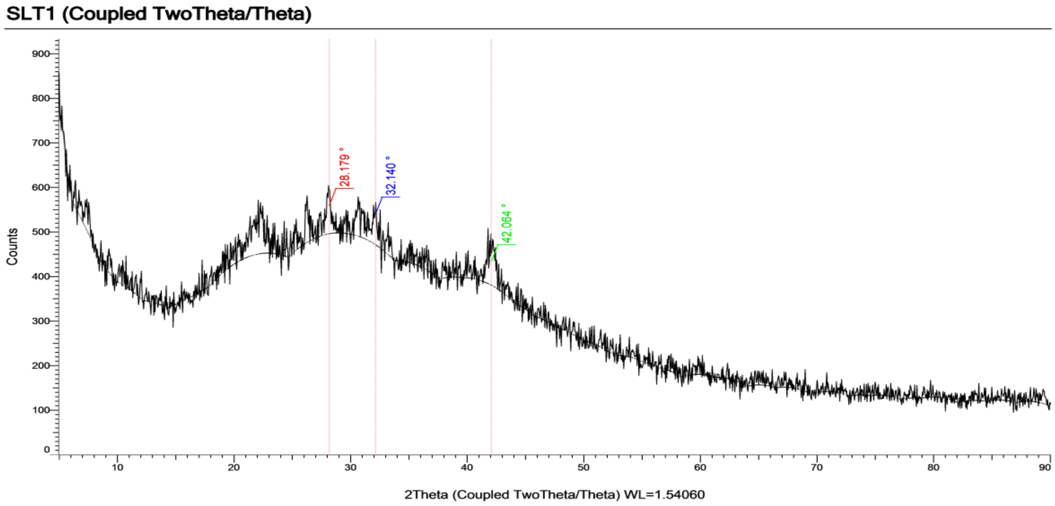


Figure 3. XRD pattern of starch based $CaCO_3$nanocomposite

3.4. TGA analysis of polymeric material

TGA curve of starch based $CaCO_3$nanocomposite hydrogel is given as Fig.4. It shows three stage decomposition. The initial stage weight (up to 10%) loss due to moisture absorbed and the second stage(at 250.10 ^oC and 360.5^oC) due to scission of amide and acrylate linkage. The third stage is decomposition of main chain. The residual weight 37.97% the charred and Ca O content

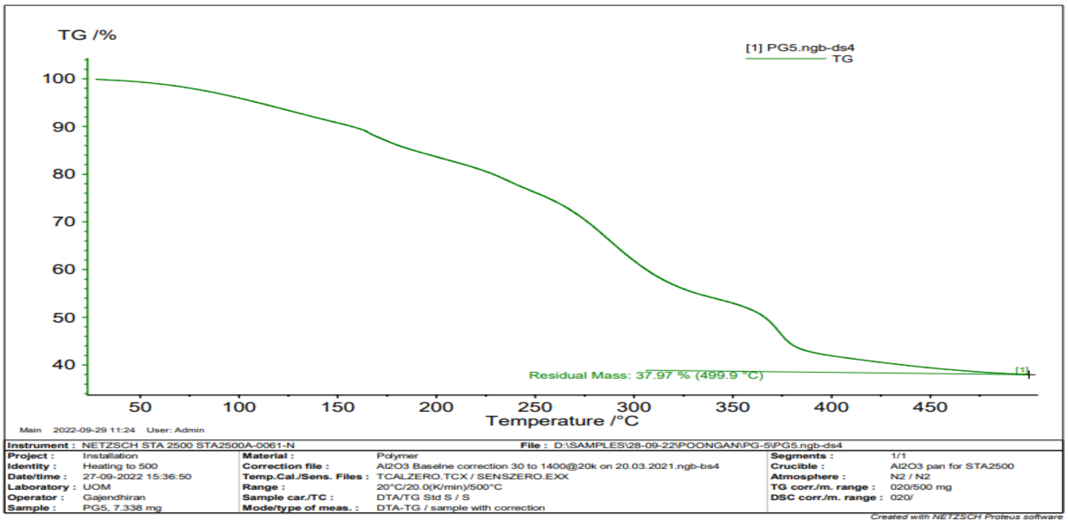


Figure 4.TGA curve of starch based $CaCO_3$nanocomposite

3.4. Antioxidant activity of starch based $CaCO_3$nanocompositehydrogel

Scavenging effect of hydrogels is given in Table 2 and Fig.5. The percentage inhibition gets increased with increase in concentration of hydrogel. Though the DPPH scavenging ability of Starch based $CaCO_3$nanocomposite was lower to that of commercially available Ascorbic acid, the research demonstrated that Starch based $CaCO_3$nanocomposite possess the capability to donate a proton and can function as a free radical inhibitor, thereby acting as primary antioxidants. From results, it is found that the Starch based $CaCO_3$hydrogels displayed strong antioxidant properties [16].

Table 2.Antioxidant activity of Starch based $CaCO_3$nanocomposite

S. No.	Conc. (μg/ml)	% of inhibition	
		Test	Standard
1	20	5.9287	22.3788
2	40	16.8965	31.6429
3	60	30.9679	43.8248
4	80	44.8755	48.5285
5	100	53.6573	63.6762
	IC_{50}	76.7633	61.0976

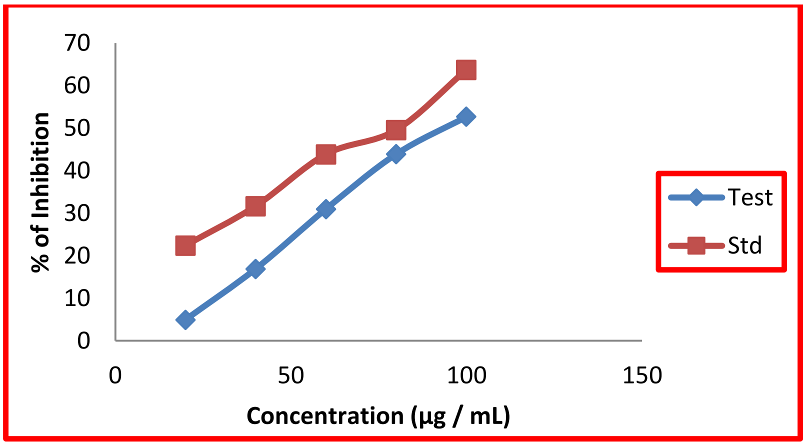


Fig.5.Antioxidant activity of Starch based $CaCO_3$ NPs composite

3.6. Antimicrobial studies Starch based $CaCO_3$hydrogels

The Table 3 and Fig.7 list the zone of inhibition of hydrogels against the micro-organism such as *Staphylococcus aureus*, *Bacillus substilis*(Gram +ve), *Escherichia coli, Salmonella paratyphi*(Gram –ve) The results regarding the zone of inhibition suggests that the antibacterial activity of the compound is specific to the targeted microorganism. The data highlights that all hydrogels exhibit relatively high inhibition values, with the exception of *Escherichia coli*, showed lower inhibition comparatively. The outcomes of the antifungal activity are presented in Table 4 and depicted in Fig.8 the zone of inhibition demonstrates that the antifungal activity of the compounds is selective, depending on the specific microorganism examined. The finding from the biological assay suggests that the antibacterial action attributed to all the compounds can be attributed to the presence of starch and cyclohexyl groups. Therefore, Starch based $CaCO_3$nanocomposite may be used for biomedical applications [14-17].

Table 3. Antibacterial activity Starch based $CaCO_3$nanocomposite

S.No	Organisms	Zone of Inhibition(mm)			
		Std. Ciprofloxacin (10µg/disc)	Samples (100µg/disc)		
			0.1 g	0.3 g	0.5 g
1.	*Staphylococcus aureus*	19	23	21	24
2.	*Bacillus subtilis*	19	17	21	22
4.	*Escherichia coli*	22	17	15	16
5.	*Salmonella paratyphi*	25	21	20	24

Table 4. Antifungal activity Starch based $CaCO_3$nanocomposite

S.No	Organisms	Zone of Inhibition(mm)			
		Std. Clotrimazole (10µg/disc)	Samples (100µg/disc)		
			0.1 g	0.3 g	0.5 g
1.	*Candida albicans*	38	28	24	32
2.	*Aspergillusniger*	32	30	35	38
3.	*Monoscuspurpures*	41	32	25	36

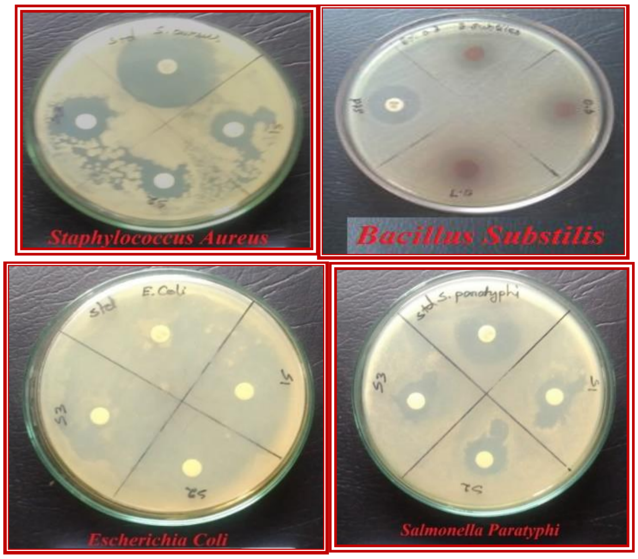


Figure 7. Antibacterial activity Starch based $CaCO_3$nanocomposite

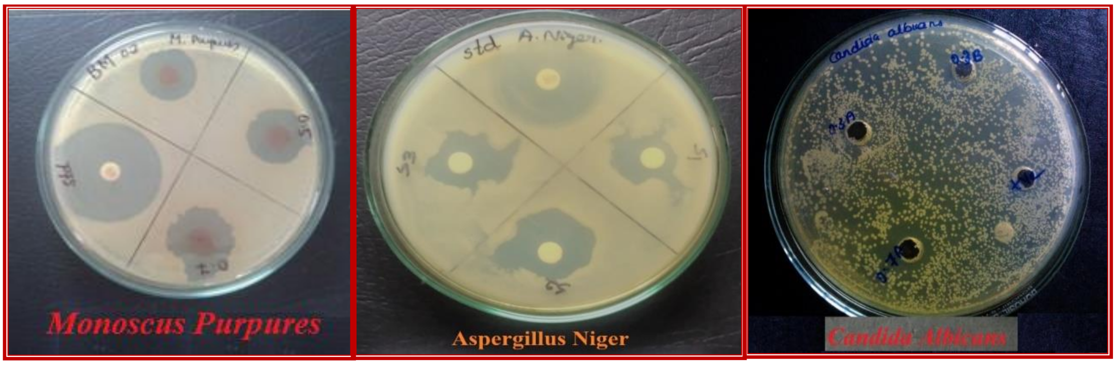


Figure 8. Antifungal activity Starch based $CaCO_3$nanocomposite

4. CONCLUSION

The $CaCO_3$ intercalated Starch based nanocomposite hydrogel was synthesized by free radical polymerization using SMA and NCA monomers. The synthesized nanocomposite hydrogels were characterized by FTIR, spectroscopy. SEM analysis indicates the surface morphology of materials showed palm leaf like structure. EDAX indicated the intercalation of $CaCO_3$ NPs in the matrix . TGA analysis showed that the stability of the materials. The antimicrobial analysis exhibited the suitability of biomaterials for medical applications.

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